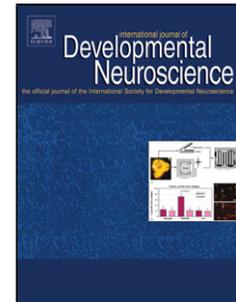


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The effect of selective opioid receptor agonists and antagonists on epileptiform activity in morphine-dependent infant mice hippocampal slices

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HIGHLIGHTS

- Excitatory effect of opiate on epileptiform activity is mediated through K receptor
- Inhibitory effect of opiate on epileptiform activity is mediated via the μ receptor
- The pattern of effect is similar in normal and morphine-dependent slices
- But the intensity of the effect is significantly stronger in normal mice
- Exposure to morphine in neonatal period alters brain susceptibility to seizure later in life

Abstract

Hippocampal slices of mouse brain were used to estimate how selective agonist and antagonist of opioid receptors alter Low-Mg⁺² artificial cerebrospinal fluid (LM-ACSF)-induced epileptiform

activities in normal and morphine-dependent mice. Brain slices were obtained from control and morphine-dependent mice. The morphine-dependent group received morphine once a day for 5 consecutive days, and the control group received saline. All injections were administered subcutaneously (s.c) in a volume of 0.1 mL on postnatal days 14-18. Brain slices were perfused with LM-ACSF along with selective agonist and antagonist of μ , κ and δ opioid receptors. Changes in spike count per unit of time were used as indices to quantify the effects of LM-ACSF exposure in the slices. In both groups, DAMGO (selective μ opioid receptor agonist) and DPDPE (selective δ opioid receptor agonist) suppressed while Dyn-A (selective κ opioid receptor agonist) potentiated the epileptiform activity. Meanwhile, BFN-A (selective μ opioid receptor antagonist) recovered epileptiform activity in normal brain slices but not in morphine-dependent ones. NTI (selective δ opioid receptor antagonist) and nor-BNI (selective κ opioid receptor antagonist) decreased epileptiform activity. It seems that the excitatory effect of morphine on epileptiform activity was mediated through kappa receptors and its inhibitory effect was mediated via the mu receptor and, to a lesser degree, through the delta receptor. The pattern of effect was similar in normal and morphine-dependent slices, but the intensity of the effect was significantly stronger in normal mice. Finding of this study might be considered for further research and attention in epilepsy treatment.

Keywords: acute; chronic; developing brain; hippocampal slices; morphine; seizure

1. Introduction

Early-life seizures have been associated with later cognitive dysfunction, depending on their type, duration, and frequency as well as the age of onset (Mikroulis & Psarropoulou, 2012). Epilepsy is a common neurological disorder affecting over 1% of the adult population (Hauser & Hesdorffer, 1990) and is associated with long-lasting neuronal plasticity changes that have been well documented in humans and replicated in a variety of animal models (Scharfman, 2002;

Spencer, 2002). Because epileptic brain tissue is unique and manifests long-lasting alterations, it has been recommended that models of epileptic tissue be employed to test anticonvulsant agents and study the mechanisms of epileptogenesis (Stables et al., 2002). Opioids appear to have a complex influence on neuronal excitability. The pro- or anti-convulsant activity of an opioid compound depends not only on its affinity to specific opioid receptor, but also on the dose, route of administration, and animal model (Saboory et al., 2007; Saboory, Gholami, Zare, & Roshan-Milani, 2014). Generally, opioids tend to inhibit neuronal activity. The stimulation of postsynaptic μ opioid receptors (MORs) activates potassium channels and inhibits voltage-dependent calcium channels, leading to hyperpolarization. Accordingly, some early studies showed that morphine suppresses audiogenic seizures and delays the onset of pentetrazole- and flurothyl-induced convulsions (Frenk, Engel, Ackermann, Shavit, & Liebeskind, 1979; Thompson, Levitt, & Stanwood, 2009). When doses which are much higher than those producing analgesia are used, morphine and related opiates may produce convulsions (H. Frenk, 1983; Frenk, Liban, Balamuth, & Urca, 1982). Meanwhile, morphine-pretreated animals show a faster acquisition of seizure activity (Hofmann, Tangri, Lafontaine, & Postuma, 2006). It has also been reported that early exposure to chronic morphine in infant rats might change their susceptibility to PTZ-induced seizure in an age-dependent manner (Gholami & Saboory, 2013; Gholami, Saboory, Zare, Roshan-Milani, & Hajizadeh-Moghaddam). The mechanism of opioid-induced epileptic activity was partially elucidated. For instance, it has been hypothesized that opioids acting on MOR inhibit the hippocampal GABA release, causing the disinhibition of pyramidal cells and epileptic activity. Indeed, an immunohistochemical study has confirmed that MORs are localized mainly on hippocampal interneurons, innervating pyramidal cell distal dendrites (Drake & Milner, 2002). In contrast, prodynorphin peptides released from granule cells during seizures decrease excitatory transmission by activating presynaptic κ opioid receptors (KORs) on perforant path neuronal terminals (Simmons, Terman, Drake, & Chavkin, 1994). Taking into

account the hyperpolarizing effect of opioids in a majority of brain regions except for the hippocampus, it has been postulated that endogenous opioid systems may play a crucial role in the self-limitation of seizures (Frenk, et al., 1979; Velisek & Mares, 1992). It was found that DAMGO – a selective agonist of MOR – enhances absence epilepsy in rats, whereas the δ opioid receptor (DOR) agonist (DPDPE) showed no such effect. Interestingly, KOR agonists suppressed absence seizures in rats (Przewłocka et al., 1995). Some new facts confirm the putative anticonvulsant effects of dynorphin and other KOR agonists in self-sustaining status epilepticus in rats (Mazarati & Wasterlain, 2002) and ethanol withdrawal seizures in mice (Beadles-Bohling & Wiren, 2006). Other studies reported somewhat different roles for opioid receptors in various models of seizures (Feng et al., 2012; Saboory, et al., 2007). Moreover, the differential effect of these receptors on seizure is not clear in normal and morphine-dependent subjects, particularly in developing brain. The purpose of this study was to evaluate the probable pro-seizure and/or anti-seizure effects of selective agonist and antagonist of opioid receptors on experimental epileptiform activity induced by LM-ACSF on hippocampal slices of normal and morphine-dependent infant mice.

2. Materials and Methods

Ingredients for the preparation of artificial cerebrospinal fluid, such as NaCl, NaH₂PO₄, NaHCO₃, KCl, MgSO₄, CaCl₂ and glucose, were purchased from Merck (Germany). Opioid receptor agonists (μ , δ , and κ , respectively) [D-Ala₂, N-Me-Phe₄, Gly₅-O₁]-enkephalin (DAMGO), [D-pen]-enkephalin (DPDPE) and dynorphin-A; and antagonists including B-funaltrexamine hydrochloride (B-FNA), naltrindole hydrochloride (NTI), and Nor-binaltorphimine dihydrochloride (nor-BNI) were prepared from Bachem (Switzerland). Morphine sulfate was purchased from the Food and Drug Department of Urmia University of Medical Sciences (Urmia, Iran). Drugs were dissolved in LM-ACSF to designated concentrations

(10 μ M). During electrophysiological recordings, all the drugs were applied via the perfuse (10 μ M), dissolved in LM-ACSF (Gáll, Orbán-Kis, & Szilágyi, 2015).

2.1 Animals

The mice were maintained at 22 ± 2 °C under a 12-h light/dark cycle (light on at 0700 h) and given food and water *ad libitum*. All experimental protocols and procedures complied with the guidelines of the 1975 Declaration of Helsinki, as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Iran, and were approved by the Research and Ethics Committee of Urmia University of Medical Sciences. All efforts were made to minimize the number of animals used. Studies were performed using male and female mice ($n = 70$) on postnatal day 14 (P14). Pups were kept in the animal house in a single cage with their mothers. On P14, the pups were randomly assigned to morphine-treated ($n=35$) or a saline-treated control groups ($n=35$). All injections were administered subcutaneously (s.c.) in a volume of 0.1 mL. The control group received daily injections of 0.9% physiological saline, and the morphine-treated group were injected with additive doses of morphine sulfate solution once a day for 5 consecutive days (2, 4, 8, 16, and 32 mg/kg) at 0800 hour. Although most previous studies had used higher doses of morphine (>32 mg/kg), we avoided such doses because they resulted in considerable toxicity symptoms and mortality in this study and our previous investigation (Saboori, Ghazizadeh, Heshmatian, & Khademansari, 2012).

2.2 Slice preparation

Mice pups ($n = 70$), aged 19–20 days (P19–20), were anesthetized with ether and quickly decapitated, and the brain was rapidly removed and transferred into ice-cold 2–5°C, continuously oxygenated with 95% O₂ – 5% CO₂ ACSF-composition (mM): 123 NaCl; 2.5 KCl; 1.5 CaCl₂; 2 MgSO₄; 25 NaHCO₃, 1.25 NaH₂PO₄, and 10 glucose (pH 7.35–7.45). The frontal lobe and the cerebellum were cut. Then, the obtained block was glued with cyanoacrylate to a Vibratome

stage (Vibroslice, Campden instruments, UK) and submerged in ice-cold oxygenated ACSF and then sliced. The experiments were performed on horizontal hippocampal slices (400 μm) for LM-ACSF-induced seizure-like events (SLEs) and interictal bursts. The slices were incubated for at least 1.5 h at room temperature in oxygenated normal artificial cerebrospinal fluid (nACSF) (Gáll, et al., 2015).

2.3 Electrophysiological recordings

Local field potential (LFP) recordings were performed with microelectrodes (3–8 $\text{M}\Omega$) pulled from glass capillaries (TW150F-4; World Precision Instruments (WPI), Sarasota, FL, USA) using a one-stage pipette puller (Narishige, Japan), filled with nACSF, and positioned in the stratum pyramidale of hippocampal area CA₁ with a manual micromanipulator (WPI, Sarasota, FL, USA). Recordings were started after 10 min of accommodation in the interfaced-type chamber with the sampling rate of 1 kHz. An amplifier (ScienceBeam, Tehran, Iran) with a preamplifier was used (bandwidth 0.16 Hz–2 kHz, 2k gain) and a built-in 50Hz notch filter. Data were digitized with the eTrace Analyzer software (ScienceBeam, Tehran, Iran) and stored on a computer hard disk for offline analysis. Recordings were performed from the CA1 pyramidal neuronal layer of the hippocampus. LM-ACSF was utilized to induce SLEs; and extracellular glass electrodes containing nACSF were positioned at locations yielding maximal field potential amplitudes in CA1 stratum pyramidale. All single-site recordings of SLEs were obtained from CA1 stratum pyramidale, filtered with 1Hz low-pass and 1000Hz high-pass filters unless stated otherwise, amplified, and recorded with an electromodule R12 and DAM80 amplifier (Axon Instruments, Foster City, CA).

2.4 Data analysis

Data distribution was checked using SPSS 22. The data that were not normally distributed were analyzed using nonparametric tests. The Wilcoxon test was employed for two dependent-group comparisons and Friedman test was used for multiple dependent comparisons. The results were expressed as mean \pm SEM, and $P < 0.05$ was considered significant.

3. Results

In the majority of brain slices, perfusion with LM-ACSF elicited the status of recurrent discharges or status-like activity. The status of recurrent discharges consisted of short 1–10s or less-than-1s discharges with a negative discharges' shift repeated every 2–5 s and lasted as long as the $[Mg^{2+}]$ in the ACSF remained low. In this study, the effects of selective agonist and antagonist of opioid receptors on SLEs in control and morphine-dependent mice varied greatly depending on their type. These results are summarized in Fig 1-6 where it can be seen that mu, kappa, and delta opioid receptor agonists and antagonists were generally effective in evoking SLEs when perfused on hippocampal slices. In each of the 72 different brain slices, status epileptiform activity were consistent and did not change their electromorphologic appearance. Status epileptiform activity in slices prepared from normal mice started approximately 5–10 min after the administration of LM-ACSF and lasted for approximately 95–120 min in a consistent and stable manner. However, in morphine-dependent slices, the activity started approximately 10–20 min after the administration of LM-ACSF and lasted as long as the $[Mg^{2+}]$ in the ACSF remained low. Meanwhile, spike counts per time unit in normal brain slices were significantly higher than those of morphine-dependent slices.

Opioid receptor selective agonists and antagonists showed various effects in the concentration and duration used in this test. The MOR agonist (DAMGO) resulted in complete inhibition of SLEs created with LM-ACSF in slices from normal and morphine-dependent mice. Nevertheless, the

selective antagonist of MOR (B-FNA) re-induced SLEs in normal mice, but suppressed this activity in morphine-dependent ones (Figs. 1 and 2).

Selective DOR agonist (DPDPE) decreased SLEs both in control and morphine-dependent brain slices. Selective DOR antagonist (NTI) decreased SLEs, but this suppression was not significant (Figs. 3 and 4).

The KOR agonist (dynorphin-A) and KOR antagonist (Nor-BNI) respectively increased and decreased SLEs both in the brain slices of normal and morphine-dependent mice (Figs. 5 and 6).

4. Discussion

In this study, the effects of selective agonists and antagonists of μ , κ and δ opioid receptors on LM-ACSF-induced SLEs were recorded in CA1 stratum pyramidale of hippocampal slices in normal and morphine-dependent infant mice. The present study confirms and further extends the existing findings by demonstrating that DAMGO and DPDPE selective agonists of MOR and DOR, respectively, are anti-seizure in mice, while Dyn-A selective agonist of KOR is convulsant. Furthermore, the selectivity of their anticonvulsant actions at KOR was demonstrated using nor-BNI selective KOR antagonist. Our results demonstrated that DAMGO and DPDPE would protect mice against convulsions, and that these effects could not be selectively blocked by their respective opioid antagonists such as B-FNA and NTI, respectively. This suggests that both MOR and DOR mediate the anticonvulsant properties of these ligands in this study, similar to flurothyl seizure models. These data are reinforced by other observations that MOR and DOR are involved in the opioid control of seizure protection. More recently, the results of experiments using the above-mentioned agonist and antagonist of opioid receptors showed that MOR and KOR have a pro-seizure effect and DOR has no effect (Saboory, et al., 2007; Thompson, et al., 2009); MOR and KOR have a pro-seizure effect and DOR has an anti-seizure effect (Feng, et al., 2012); and

MOR and DOR have a pro-seizure effect and KOR has an anti-seizure effect (Przewłocka, et al., 1995; Yajima et al., 2000).

It has been reported that the chronic use of opioid receptor agonists such as morphine leads to remarkable down-regulation of corresponding receptors, which is one of the mechanisms of tolerance to opiates (Potschka, Friderichs, & Löscher, 2000). Meanwhile, it was reported that morphine-pretreated animals show a faster acquisition of seizure activity. The evaluation of the postictal seizure suppression after a fully kindled seizure demonstrates that morphine-pretreated rats have a decreased sensitivity to subsequent kindling stimulations (Hofmann, et al., 2006). It has also been reported that early exposure to chronic morphine in infant rats might change their susceptibility to PTZ-induced seizure in an age-dependent manner (Gholami & Saboory, 2013; Gholami, Saboory, Zare, et al.). Previous studies have demonstrated that chronic opiate exposure during infancy affects the developing brain and alters the number and sensitivity of opioid receptors throughout life (Thornton & Smith, 1998). Clinical studies have reported an increased prevalence of autonomic dysregulation, nystagmus, and strabismus in children exposed prenatally to opiates. At the pre- and elementary school ages, these children show motor and cognitive impairments, inattention, and hyperactivity (Ross, Graham, Money, & Stanwood, 2015). Repeated morphine exposure early in life could result in long-term alterations in the opioid response (Rozisky et al., 2008). Opioid receptor expression undergoes significant developmental regulation. Some studies have suggested that opioid receptor development occurs within the first 2 weeks of life (Nandi & Fitzgerald, 2005), and that MOR density and binding peaks at P7 and subsequently decreases to adult levels (Rahman, Dashwood, Fitzgerald, Aynsley-Green, & Dickenson, 1998). Chronic administration of morphine between postnatal days 1 and 8 produces a marked decrease in the number of brain MORs, which then gradually increases to control levels by day 14 of treatment. Longer treatment cannot produce further changes to opioid receptors. No significant changes to other receptors (δ -or κ) have been

observed (Tempel & Espinoza, 1992). In a model of morphine sensitization in rats, autoradiography revealed a significant increase in the number of MORs in the hippocampus (Vigano et al., 2003) that making this system one of the predominant endogenous modulatory mechanisms that affect seizure susceptibility. Although the hippocampal formation is a structure that expresses significant densities of opioid receptors, morphine and other neurotransmitters including glutamate, acetylcholine, GABA, and nitric oxide appear to have a functional relationships (Saboory, et al., 2014). The co-localization between hippocampal MOR and GABAergic interneurons is observed in CA1, CA3, and dentate gyrus regions in rats, suggesting that these receptors can directly control hippocampal GABAergic neuronal activity (Kalyuzhny & Wessendorf, 1998). Biochemical data indicate that acute morphine reduces synaptosomal calcium. Nevertheless, with the development of dependence, the calcium level in synaptosomes increases in a proportional way (Yang et al., 2010). Meanwhile, it has been reported that morphine, through the sustained activation of opioid receptors, can promote abnormal programmed cell death by enhancing the expression of pro-apoptotic Fas receptor protein and damping the expression of anti-apoptotic Bcl-2 oncoprotein. These changes might alter the seizure susceptibility of morphine treated mice (Boronat, García-Fuster, & García-Sevilla, 2001). Also, Chronic opiate exposure induces neuronal excitation, resulting in elevated intracellular Ca^{2+} levels, which are toxic to neurons. The pathological elevation of intracellular Ca^{2+} concentration is buffered by Ca^{2+} binding proteins such as Parvalbumin, Calretinin, Calbindin. Changes in the expression of Calcium binding proteins in specific regions of neonatal mouse brain have been reported (Maharajan et al., 2000; Mithbaokar, Fiorito, Della Morte, Maharajan, & Costagliola, 2016).

Opiate depresses the stimulated release of the excitatory transmitter by decreasing the supply of Ca^{2+} ions to the stimulus-release coupling mechanism in sympathetic nerve terminals (Illes, Zieglga, & Herz, 1980). In a study, tolerance was induced in rats by subcutaneous implantation

of slow-release pellets of 75 mg of morphine over 8 days. No increased electrographic spiking occurred in these animals when compared to non-tolerant controls. However, when these animals were injected with morphine (300 mg/kg) and withdrawal was subsequently precipitated by naltrexone, there were significantly more seizures in the tolerant animals as compared to the non-tolerant ones (Hanan Frenk, 1983). While these studies do support the hypothesis that electrographic seizures elicited by i.c.v. endorphins and, to a lesser degree, i.c.v. morphine, are mediated by specific opiate receptors, it seems that these receptors are different from the MOR assumed to mediate the analgesic effects of opiates. This observation, together with the relatively high doses of naloxone needed to reverse the epileptogenic effects of enkephalins and morphine, suggested that DOR mediates these effects (Hanan Frenk, 1983). We found that pretreatment with systemic morphine delayed the onset and reduced the count of LM-ACSF-induced SLEs. It has been reported that morphine delays the onset of generalized myoclonus in the photosensitive baboon and naloxone reverses this effect (Hofmann, et al., 2006). In a study similar to ours, the use of morphine during P8–P14 reduced seizure behavior and severity in adults, representing the long-term, anti-convulsant effect of morphine. Therefore, the result of the present study is consistent with findings of other investigations.

Both anti- and pro-convulsant effects of morphine in chemical and electrical seizure models can be reversed by MOR antagonists (Lauretti, Ahmad, & Pleuvry, 1994). This effect was not observed in the present study by B-FNA selective MOR antagonist in morphine-dependent brain slices but accrued in the normal group. It is possible that the mechanism of this action is mediated through inhibitory effects on glutamatergic excitatory pathways and increasing the GABAergic activity (Zieglansberger, French, Siggins, & Bloom, 1979). It has also been demonstrated that prenatal morphine exposure causes an up-regulation of $\alpha 1$ subunit and down-regulation of $\beta 2/\gamma 2$ subunit expression of GABA A receptors, associated with an increase in seizure susceptibility (Wang et al., 2011). In our study, it is therefore possible that following

repeated early-life morphine exposure, alteration or desensitization of MOR might have led to the alteration of GABAergic transmission in the hippocampus, which may, in turn, have caused the anti-convulsant effects of mu and delta agonist and resulted in the suppression of GABAergic interneurons that decrease GABA release (Saboory, et al., 2007). As was observed in this study, KOR may be involved in the excitatory effect of morphine on SLEs. Although the low Mg^{2+} /high K^+ model-induced SLEs cannot fully reflect the mechanisms of epileptic syndromes, it is the most popular technique to obtain SLEs. The ultimate goal of this study was the analysis of SLEs by spike count which characterizes the pharmacologic action of opioid receptors' agonists and antagonist in normal and morphine-dependent infant mice. The pro- and anti-seizure actions of drugs were analyzed by evaluating the total suppression of SLEs. Based on the pattern of modifications, the pharmacological actions were sometimes pro-seizure rather than anti-seizure (Gáll, et al., 2015). The presynaptic inhibitory effect of morphine is likely induced by the reduction of Ca^{2+} entrance into nerve terminals and, thereby, through the prevention of the release of glutamate in the cerebral cortex (Castillo, Chiu, & Carroll, 2011). In addition, it is possible that the activation of NO system by different doses of morphine can lead to opposite effects on seizure susceptibility, and that NO might induce the anticonvulsant effect of morphine by an increase in GABAergic tone (Gholami, Saboory, & Roshan-Milani; Homayoun, Khavandgar, Namiranian, Gaskari, & Dehpour, 2002). Moreover, a number of studies have shown an interaction between opioid and cholinergic systems (Walker, McGlynn, Grey, Ragozzino, & Gold, 1991) and reported the inhibitory effect of morphine on cholinergic activity in the hippocampus (Decker & McGaugh, 1991). The cholinergic activation of hippocampal circuits may recruit the opioid-mediated modulation of hippocampal network states. Results of the present study and others (Chen, Chen, & Liu-Chen, 2007) demonstrate that opioid peptides with a pharmacological selectivity for MOR and DOR are anticonvulsant, and opioid peptides with a pharmacological selectivity for KOR are convulsant. It is interesting to note that MOR and DOR selectively

hyperpolarize neurons throughout the CNS (Przewłocka, Stala, Lasoń, & Przewłocki, 1983) and that endogenous opioids are localized in fiber tracts throughout the brain and spinal cord, involved in the generation and spread of seizure activity (Klein et al., 2005). Therefore, it seems possible that opioid peptides may indeed function as endogenous anticonvulsants in the CNS, modulating the underlying mechanisms of seizure arrest and refractoriness which are critical to the suppression of convulsions. When we put all these results together, it appears that the administration of systemic morphine in the absence of additional convulsant manipulations activates at least two epileptogenic mechanisms one of which is mediated by specific opiate receptors, while the other is not. These results seem to indicate that the anticonvulsant effects of opioids are mediated by specific opiate receptors which are probably MOR and DOR and their convulsant effects occur via KOR. We strongly encourage increased attention to the potential effects of prenatal opiate exposure on brain development. Current data are scarce and partly complicated by interpretational problems. This should motivate further investigation of the effects of prenatal opiate exposure in the brain, so that health-care providers and policy makers can be sufficiently informed when making treatment programs for pregnant opioid dependent women and their children (Thompson, et al., 2009).

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Ethical approval

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of interest statement

The authors have no conflicts of interest to declare regarding the study described in this article and the preparation of the article.

References

- Beadles-Bohling, A., & Wiren, K. (2006). Anticonvulsive effects of κ -Opioid receptor modulation in an animal model of ethanol withdrawal. *Genes Brain Behav.*, 5(6), 483-496.
- Boronat, M. A., García-Fuster, M. J., & García-Sevilla, J. A. (2001). Chronic morphine induces up-regulation of the pro-apoptotic Fas receptor and down-regulation of the anti-apoptotic Bcl-2 oncoprotein in rat brain. *British Journal of Pharmacology*, 134(6), 1263-1270. doi: 10.1038/sj.bjp.0704364
- Castillo, P., Chiu, C. Q., & Carroll, R. (2011). Long-term synaptic plasticity at inhibitory synapses. *Curr Opin Neurobiol*, 21(2), 328-338. doi: 10.1016/j.conb.2011.01.006
- Chen, Y., Chen, C., & Liu-Chen, L. (2007). Dynorphin peptides differentially regulate the human κ opioid receptor. *Life Sci.*, 80(15), 1439-1448.
- Decker, M., & McGaugh, J. (1991). The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse*, 7(2), 151-168.
- Drake, C., & Milner, T. (2002). Mu opioid receptors are in discrete hippocampal interneuron subpopulations. *Hippocampus*, 12(2), 119-136.
- Feng, Y., He, X., Yang, Y., Chao, D., Lazarus, L. H., & Xia, Y. (2012). Current Research on Opioid Receptor Function. *Curr Drug Targets*, 13(2), 230-246.
- Frenk, H. (1983). Pro- and anticonvulsant actions of morphine and the endogenous opioids: involvement and interactions of multiple opiate and non-opiate systems. *Brain Res.*, 6(2), 197-210.
- Frenk, H. (1983). Pro- and anticonvulsant actions of morphine and the endogenous opioids: involvement and interactions of multiple opiate and non-opiate systems. *Brain Research Reviews*, 6(2), 197-210.
- Frenk, H., Engel, J., Ackermann, R., Shavit, Y., & Liebeskind, J. (1979). Endogenous opioids may mediate post-ictal behavioral depression in amygdaloid-kindled rats. *Brain Res.*, 167(2), 435-440.
- Frenk, H., Liban, A., Balamuth, R., & Urca, G. (1982). Opiate and non-opiate aspects of morphine induced seizures. *Brain Res.*, 253(1), 253-261.
- Gáll, Z., Orbán-Kis, K., & Szilágyi, T. (2015). Differential effects of sodium channel blockers on in vitro induced epileptiform activities. *Arch Pharm Res.*, 1-10.
- Gholami, M., & Saboory, E. (2013). Morphine exposure induces age-dependent alterations in pentylenetetrazole-induced epileptic behaviors in prepubertal rats. *Dev Psychobiol.*, 55(8), 881-887. doi: 10.1002/dev.21080

- Gholami, M., Saboory, E., & Roshan-Milani, S. Proconvulsant effects of tramadol and morphine on pentylenetetrazol-induced seizures in adult rats using different routes of administration. *Epilepsy Behav.*, 36, 90-96. doi: 10.1016/j.yebeh.2014.05.012
- Gholami, M., Saboory, E., Zare, S., Roshan-Milani, S., & Hajizadeh-Moghaddam, A. The effect of dorsal hippocampal administration of nicotinic and muscarinic cholinergic ligands on pentylenetetrazol-induced generalized seizures in rats. *Epilepsy Behav.*, 25(2), 244-249. doi: 10.1016/j.yebeh.2012.07.004
- Hauser, W., & Hesdorffer, D., 1990. *Epilepsy: frequency, causes and consequences* (Vol. 376): Demos Medical Pub.
- Hofmann, A., Tangri, N., Lafontaine, A. L., & Postuma, R. B. (2006). Myoclonus as an acute complication of low-dose hydromorphone in multiple system atrophy. *J Neurol Neurosurg Psychiatry* 77(8), 994-995. doi: 10.1136/jnnp.2005.076588
- Homayoun, H., Khavandgar, S., Namirani, K., Gaskari, S. A., & Dehpour, A. (2002). The role of nitric oxide in anticonvulsant and proconvulsant effects of morphine in mice. *Epilepsy Res.*, 48(1-2), 33-41. doi: [http://dx.doi.org/10.1016/S0920-1211\(01\)00316-3](http://dx.doi.org/10.1016/S0920-1211(01)00316-3)
- Illes, P., Zieglga, W., & Herz, A. (1980). Calcium reverses the inhibitory action of morphine on neuroeffector transmission in the mouse vas deferens. *Brain Res.*, 191(2), 511-522.
- Kalyuzhny, A., & Wessendorf, M. (1998). Relationship of μ - and δ -opioid receptors to GABAergic neurons in the central nervous system, including antinociceptive brainstem circuits. *J Comp Neurol.*, 392(4), 528-547.
- Klein, M., Rudich, Z., Gurevich, B., Lifshitz, M., Brill, S., Lottan, M., & Weksler, N. (2005). Controlled-release oxycodone-induced seizures. *Clin Ther.*, 27(11), 1815-1818.
- Lauretti, G., Ahmad, I., & Pleuvry, B. (1994). The activity of opioid analgesics in seizure models utilizing N-methyl-DL-aspartic acid, kainic acid, bicuculline and pentylenetetrazole. *Neuropharmacology*, 33(2), 155-160.
- Maharajan, P., Prencipe, R., Di Francesco, P., Paino, G., Ravagnan, G., & Maharajan, V. (2000). Maternal morphine alters parvalbumin immunoreactivity patterns in neonatal mouse brain. *Synapse*, 35(4), 265-271. doi: 10.1002/(SICI)1098-2396(20000315)35:4<265::AID-SYN4>3.0.CO;2-6
- Mazarati, A., & Wasterlain, C. (2002). Anticonvulsant effects of four neuropeptides in the rat hippocampus during self-sustaining status epilepticus. *Neurosci Lett.*, 331(2), 123-127.
- Mikroulis, A., & Psarropoulou, C. (2012). Endogenous ACh effects on NMDA-induced interictal-like discharges along the septotemporal hippocampal axis of adult rats and their modulation by an early life generalized seizure. *Epilepsia*, 53(5), 879-887.
- Mithbaokar, P., Fiorito, F., Della Morte, R., Maharajan, V., & Costagliola, A. (2016). Chronic maternal morphine alters calbindin D-28k expression pattern in postnatal mouse brain. *Synapse*, 70(1), 15-23. doi: 10.1002/syn.21866
- Nandi, R., & Fitzgerald, M. (2005). Opioid analgesia in the newborn. *Eur J Pain.*, 9(2), 105-108.
- Potschka, H., Friderichs, E., & Löscher, W. (2000). Anticonvulsant and proconvulsant effects of tramadol, its enantiomers and its M1 metabolite in the rat kindling model of epilepsy. *Br J Pharmacol.*, 131(2), 203-212. doi: 10.1038/sj.bjp.0703562
- Przewłocka, B., Lasoń, W., Machelska, H., Van Luijtelaa, G., Coenen, A., & Przewłocki, R. (1995). Kappa opioid receptor agonists suppress absence seizures in WAG/Rij rats. *Neurosci Lett.*, 186(2), 131-134.
- Przewłocka, B., Stala, L., Lasoń, W., & Przewłocki, R. (1983). The effect of various opiate receptor agonists on the seizure threshold in the rat. Is dynorphin an endogenous anticonvulsant? *Life Sci.*, 33, 595-598.
- Rahman, W., Dashwood, M., Fitzgerald, M., Aynsley-Green, A., & Dickenson, A. (1998). Postnatal development of multiple opioid receptors in the spinal cord and development of spinal morphine analgesia. *Brain Res Dev Brain Res.*, 108(1), 239-254.

- Ross, E. J., Graham, D. L., Money, K. M., & Stanwood, G. D. (2015). Developmental Consequences of Fetal Exposure to Drugs: What We Know and What We Still Must Learn. *Neuropsychopharmacology*, *40*(1), 61-87. doi: 10.1038/npp.2014.147
- Rozisky, J., Dantas, G., Adachi, L., Alves, V., Ferreira, M., Sarkis, J., & Torres, I. (2008). Long-term effect of morphine administration in young rats on the analgesic opioid response in adult life. *Int J Dev Neurosci.*, *26*(6), 561-565.
- Saboory, E., Derchansky, M., Ismaili, M., Jahromi, S. S., Brull, R., Carlen, P. L., & El Beheiry, H. (2007). Mechanisms of morphine enhancement of spontaneous seizure activity. *Anesth Analg*, *105*(6), 1729-1735, table of contents. doi: 105/6/1729 [pii]
- 10.1213/01.ane.0000287675.15225.0b
- Saboory, E., Ghazizadeh, V., Heshmatian, B., & Khademansari, M. (2012). Desmopressin accelerates the rate of urinary morphine excretion and attenuates withdrawal symptoms in rats. *Psychiatry Clin Neurosci.*, *66*(7), 594-601.
- Saboory, E., Gholami, M., Zare, S., & Roshan-Milani, S. (2014). The long-term effects of neonatal morphine administration on the pentylenetetrazol seizure model in rats: The role of hippocampal cholinergic receptors in adulthood. *Dev Psychobiol.*, *56*(3), 498-509.
- Scharfman, H. (2002). Book review: Epilepsy as an example of neural plasticity. *The Neuroscientist*, *8*(2), 154-173.
- Simmons, M., Terman, G., Drake, C., & Chavkin, C. (1994). Inhibition of glutamate release by presynaptic kappa 1-opioid receptors in the guinea pig dentate gyrus. *J Neurophysiol.*, *72*(4), 1697-1705.
- Spencer, S. (2002). Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia*, *43*(3), 219-227.
- Stables, J., Bertram, E., White, H., Coulter, D., Dichter, M., Jacobs, M., . . . Noebels, J. (2002). Models for epilepsy and epileptogenesis: report from the NIH workshop, Bethesda, Maryland. *Epilepsia*, *43*(11), 1410-1420.
- Tempel, A., & Espinoza, K. (1992). Morphine-induced Downregulation of μ -Opioid Receptors and Peptide Synthesis in Neonatal Rat Brain. *Ann N Y Acad Sci.*, *654*(1), 529-530.
- Thompson, B., Levitt, P., & Stanwood, G. (2009). Prenatal exposure to drugs: effects on brain development and implications for policy and education. [10.1038/nrn2598]. *Nat Rev Neurosci*, *10*(4), 303-312.
- Thornton, S., & Smith, F. (1998). Long-term alterations in opiate antinociception resulting from infant fentanyl tolerance and dependence. *Eur J Pharmacol.*, *363*(2), 113-119.
- Velisek, L., & Mares, P. (1992). Differential effects of naloxone on postictal depression. *Epilepsy Res.*, *12*(1), 37-43.
- Vigano, D., Rubino, T., Di Chiara, G., Ascari, I., Massi, P., & Parolaro, D. (2003). μ opioid receptor signaling in morphine sensitization. *Neuroscience*, *117*(4), 921-929.
- Walker, D., McGlynn, T., Grey, C., Ragozzino, M., & Gold, P. (1991). Naloxone modulates the behavioral effects of cholinergic agonists and antagonists. *Psychopharmacology*, *105*(1), 57-62.
- Wang, C., Hung, C., Lin, C., Lee, H., Yang, C., Jong, Y., & Yang, S. (2011). Differential alterations of GABA A receptor (α 1, β 2, γ 2 subunit) expression and increased seizure susceptibility in rat offspring from morphine-addicted mothers: Beneficial effect of dextromethorphan. *Neurosci Lett.*, *489*(1), 5-9.
- Yajima, Y., Narita, M., Takahashi-Nakano, Y., Misawa, M., Nagase, H., Mizoguchi, H., . . . Suzuki, T. (2000). Effects of differential modulation of μ -, δ -and κ -opioid systems on bicuculline-induced convulsions in the mouse. *Brain Res*, *862*(1), 120-126.
- Yang, L., Li, F., Ge, W., Mi, C., Wang, R., & Sun, R. (2010). Protective effects of naloxone in two-hit seizure model. *Epilepsia*, *51*(3), 344-353.

Zieglansberger, W., French, E. D., Siggins, G. R., & Bloom, F. E. (1979). Opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons. *Science*, 205(4404), 415-417.

Figure 1: Graphical illustration of selective MOR agonist (DAMGO) and antagonist (B-FNA) effects on LM-ACSF-induced SLEs in the brain slices of normal and morphine-dependent mice. **A:** SLEs recorded from CA1 area of hippocampus perfused with LM-ACSF; **B:** DAMGO 10 μ M completely depressed LM-ACSF-induced SLEs; **C:** B-FNA 10 μ M recovered the SLEs in normal slices but not in morphine-dependent ones; **D:** washout with LM-ACSF reversed the effects of drugs and re-induced the SLEs.

Figure 2: Effects of MOR agonist (DAMGO) and antagonist (B-FNA) on SLEs in normal and morphine-dependent infant mice. In both groups, DAMGO suppressed SLEs and B-FNA recovered the activity in normal but not in morphine-dependent mice, whereas washout with LM-ACSF re-induced SLEs in both groups. The experiments were performed on n=10 slices in control and n=6 slices in other stages. * indicates significant difference from control in both series ($P < 0.01$); @ indicates significant difference from DAMGO in normal slices ($p = 0.035$); # indicates significant difference from morphine-dependent slices at the same stage ($P < 0.02$).

Figure 3: Graphical illustration of DOR agonist (DPDPE) and antagonist (NTI) effects on LM-ACSF-induced SLEs in the brain slices of normal and morphine-dependent infant mice. **A:** SLEs

recorded from CA1 area of hippocampus perfused with LM-ACSF; **B**: in both groups of slices, DPDPE attenuated LM-ACSF-induced SLEs; **C**: NTI did not show any remarkable changes on SLEs in either group; **D**: washout with LM-ACSF reversed the effects of drugs.

Figure 4: Effects of DOR agonist (DPDPE) and antagonist (NTI) on SLEs in normal and morphine-dependent infant mice. In both groups, DPDPE decreased spike count number and NTI did not recover SLEs, but even potentiated the suppressive effect of DPDPE in morphine-dependent slices. However, washout with LM-ACSF recovered SLEs. The experiments were performed on n=10 slices in control and n=6 slices in other stages. * indicates significant difference from control in each group ($P<0.02$); # indicates significant difference between the two groups at each stage ($P<0.04$).

Figure 5: Graphical illustration of selective KOR agonist (Dyn-A) and antagonist (Nor-BNI) effects on LM-ACSF-induced SLEs in the brain slices of normal and morphine-dependent infant mice. **A**: SLEs recorded from CA1 area of hippocampus perfused with LM-ACSF. **B**: Dyn-A potentiated the LM-ACSF-induced SLEs; **C**: Nor-BNI decreased the spike count; **D**: washout with LM-ACSF reversed the effects of drugs.

Figure 6: Effects of selective KOR agonist (Dyn-A) and antagonist (nor-BNI) on SLEs in normal and morphine-dependent infant mice. In both groups, Dyn-A increased spike count number and nor-BNI decreased the effect of Dyn-A, whereas washout with LM-ACSF potentiated the SLEs. The experiments were performed on n=10 slices in control and n=6 slices in other stages. * indicates significant difference from control in both series; # indicates significant difference between

normal and morphine-dependent slices at each identical stage ($p < 0.02$); @ indicates significant difference from Dyn-A in both series ($P < 0.04$).

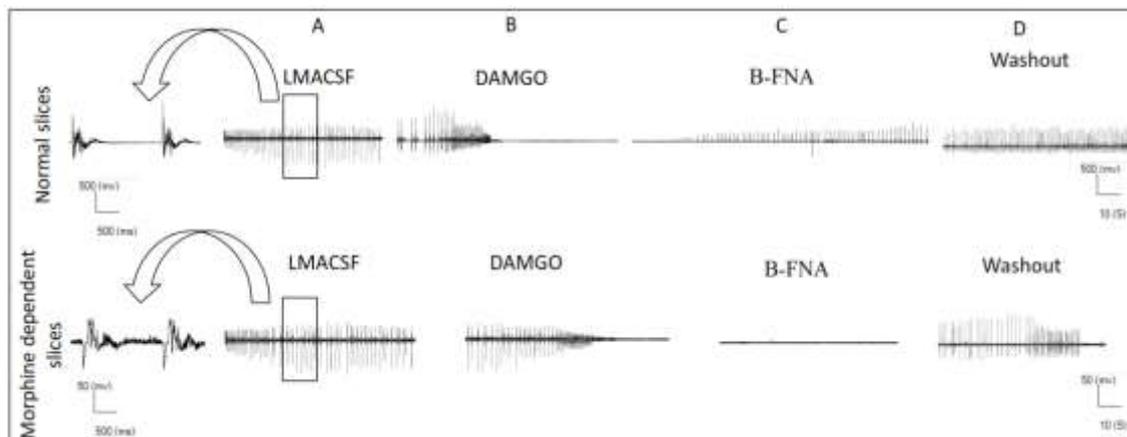


Figure 1:

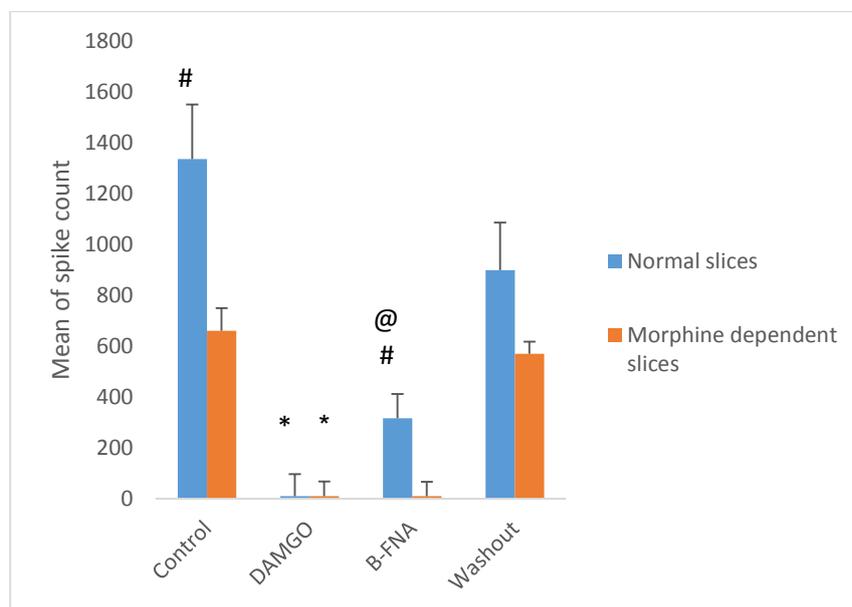


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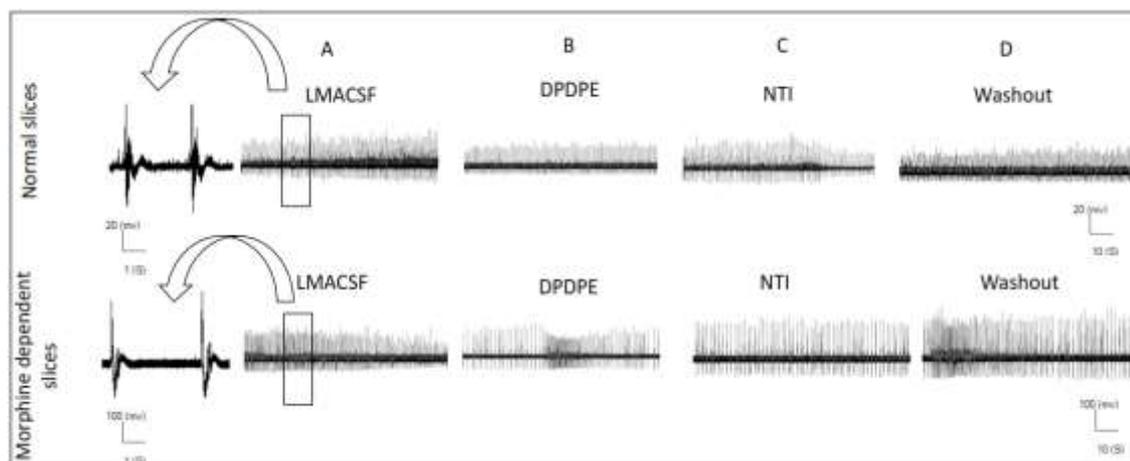


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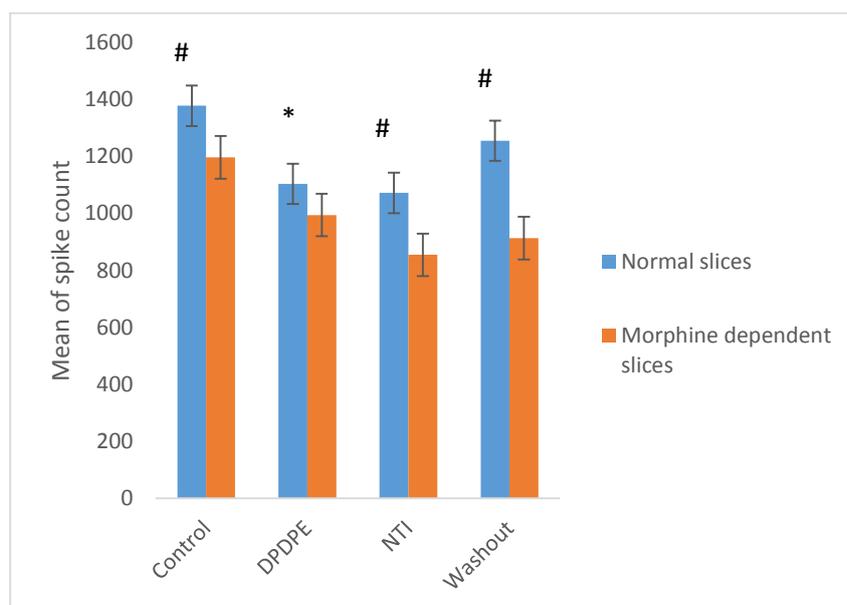


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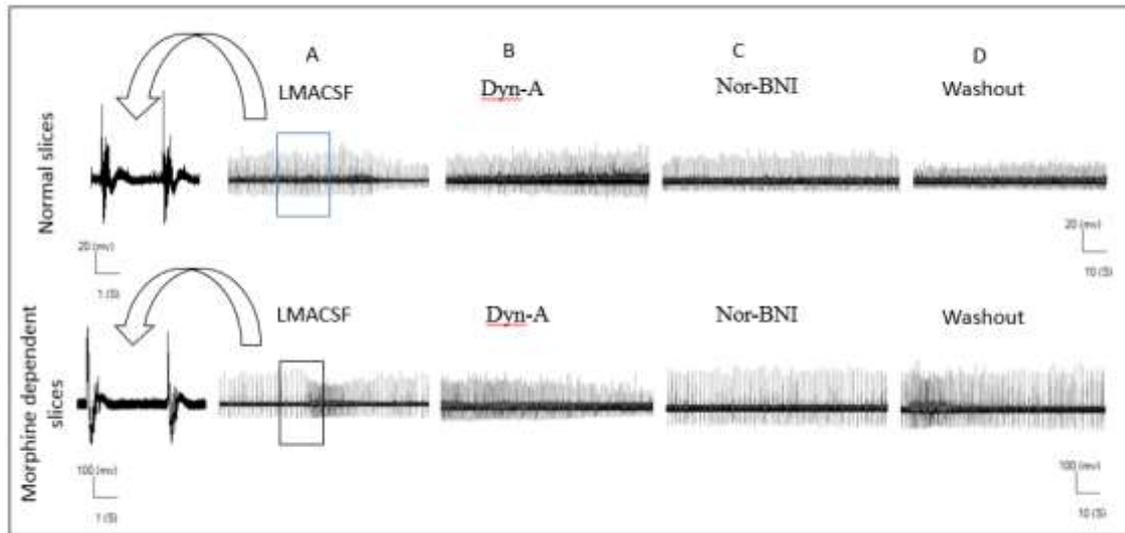


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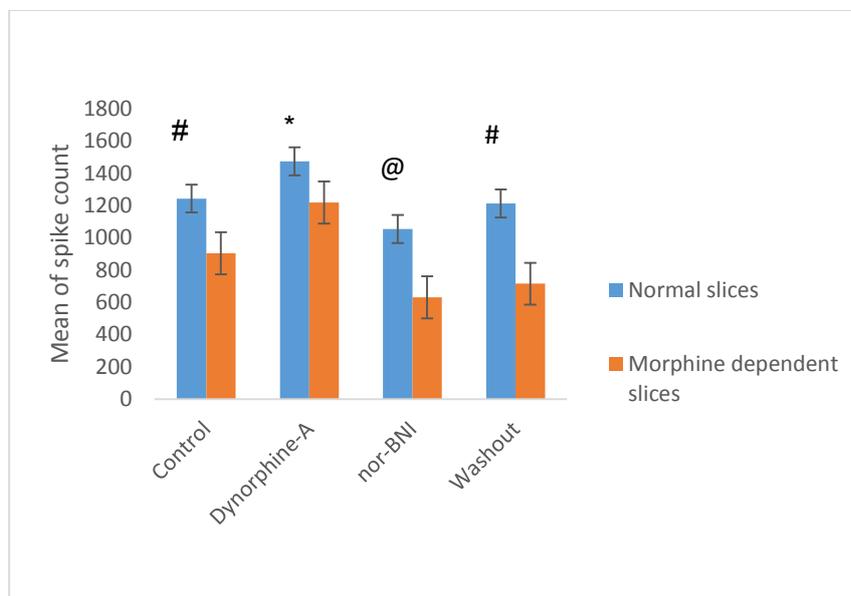


Figure 6: